



**HAL**  
open science

## Analysis of adulterated herbal medicines and dietary supplements marketed for weight loss by DOSY $^1\text{H}$ NMR

Julie Vaysse, Stéphane Balayssac, Véronique Gilard, Denis Desoubzdanne, Myriam Malet-Martino, Robert Martino

► **To cite this version:**

Julie Vaysse, Stéphane Balayssac, Véronique Gilard, Denis Desoubzdanne, Myriam Malet-Martino, et al.. Analysis of adulterated herbal medicines and dietary supplements marketed for weight loss by DOSY  $^1\text{H}$  NMR. Food Additives and Contaminants, 2010, 27 (07), pp.903-916. 10.1080/19440041003705821 . hal-00598944

**HAL Id: hal-00598944**

**<https://hal.science/hal-00598944>**

Submitted on 8 Jun 2011

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



**Analysis of adulterated herbal medicines and dietary supplements marketed for weight loss by DOSY 1H NMR**

Journal:	<i>Food Additives and Contaminants</i>
Manuscript ID:	TFAC-2009-378.R1
Manuscript Type:	Original Research Paper
Date Submitted by the Author:	14-Feb-2010
Complete List of Authors:	VAYSSE, Julie; Université Paul Sabatier, SPCMIB Lab BALAYSSAC, Stéphane; Université Paul Sabatier, SPCMIB Lab GILARD, Véronique; Université Paul Sabatier, SPCMIB Lab DESOUBZDANNE, Denis; Université Paul Sabatier, SPCMIB Lab MALET-MARTINO, Myriam; Université Paul Sabatier, SPCMIB MARTINO, Robert; Université Paul Sabatier, SPCMIB Lab
Methods/Techniques:	Analysis - NMR
Additives/Contaminants:	Additives general
Food Types:	Nutritional supplements

SCHOLARONE™  
Manuscripts

1  
2  
3  
4 **Analysis of adulterated herbal medicines and dietary supplements**  
5  
6 **marketed for weight loss by DOSY  $^1\text{H}$  NMR**  
7  
8

9  
10  
11 **Julie VAYSSE, Stéphane BALAYSSAC, Véronique GILARD,**

12  
13 **Denis DESOUBDZANNE, Myriam MALET-MARTINO\*, Robert MARTINO**

14  
15  
16 Université de Toulouse; UPS; Laboratoire de Synthèse et Physico-Chimie de Molécules  
17  
18 d'Intérêt Biologique (SPCMIB), Groupe de RMN Biomédicale; 118 route de Narbonne,  
19  
20 31062 Toulouse cedex 9, France  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34

35 **Myriam Malet-Martino\*: tel 33 5 61 55 68 90; fax 33 5 61 55 76 25**

36  
37 **[martino@chimie.ups-tlse.fr](mailto:martino@chimie.ups-tlse.fr)**  
38  
39  
40  
41  
42  
43  
44  
45

46 **Keywords:** slimming products; herbal medicines; dietary supplements; adulteration;  
47  
48 synthetic drugs;  $^1\text{H}$  NMR; 2D and 3D DOSY  $^1\text{H}$  NMR; sibutramine; phenolphthalein;  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
synephrine

**Abstract**

Twenty herbal medicines or dietary supplements marketed as natural slimming products were analyzed by Diffusion Ordered Spectroscopy (DOSY)  $^1\text{H}$  NMR and DOSY-COSY  $^1\text{H}$  NMR. The method allows analysis of the whole sample with detection of both active and inactive ingredients in these complex matrices. Among the 20 formulations analyzed, 2 were strictly herbal and 4 had a composition corresponding to declared ingredients on the packaging or the leaflet. The others were all adulterated. Eight formulations contain sibutramine alone at doses ranging from 4.4 to 30.5 mg/capsule. Five formulations contain sibutramine (from 5.0 to 19.6 mg/capsule or tablet) in combination with phenolphthalein (from 4.4 to 66.1 mg/capsule), and the last formulation was adulterated with synephrine (19.5 mg/capsule). Quantification of the actives was carried out with  $^1\text{H}$  NMR. Several other compounds were also characterized including methylsynephrine, vitaberin, sugars, vitamins etc. DOSY NMR is thus proposed as a useful tool for detection of unexpected adulteration.

## Introduction

According to WHO (<http://www.who.int/mediacentre/factsheets/fs311/en/>), approximately 1.6 billion adults were overweight and at least 400 million were obese in 2005. WHO further projects that by 2015, approximately 2.3 billion adults will be overweight and more than 700 million will be obese. Overweight and obesity are associated with premature mortality, chronic morbidity, diabetes, heart disease, osteoarthritis and cancer. Given (i) the medical and social impact of being overweight or obese, (ii) the few drugs marketed for these indications, and (iii) the safety concerns of these medications, more and more patients turn to weight-loss natural herbal medicines (HM) or dietary supplements (DS). Indeed, in contrast to conventional pharmaceuticals, these products are regarded by many as being harmless because of their natural origin for HM or of their safe composition (vitamins, minerals, herbs or other botanicals, amino acids, and substances such as enzymes, organ tissues, glandular, and metabolites) for DS.

The WHO's definition indicates that medicines containing plant material combined with chemically defined active substances, including chemically defined, isolated constituents of plants, are not considered to be HM (<http://www.who.int/medicines/areas/traditional/definitions/en/index.html>). Moreover, FDA regulations require that a complete list of ingredients and the net contents of the product appear on DS labels (<http://www.fda.gov/food/DietarySupplements/default.htm>). Despite these clear definition and regulation and as it is very easy for the consumer to purchase these formulations over the Internet, common problems affecting the safety of these preparations include fraudulent adulteration with synthetic actives used in conventional pharmaceutical drugs which can lead to severe side-effects.

Reported methods for screening illegal adulterations in HM or DS are mainly HPLC-DAD, GC-MS and LC/MS/MS (Liu et al. 2001; Lau et al. 2003; Bogusz et al. 2006; Liang et

1  
2  
3 al. 2006; Chen et al. 2009). Recently, Zhu et al. (2009) developed an algorithm, termed local  
4  
5 straight-line screening (LSLS), to resolve complex IR spectra of possibly adulterated HM.  
6  
7  
8 The analytical methods reported concerning more specifically the adulteration of herbal  
9  
10 formulations marketed for weight loss are chromatographic methods coupled with various  
11  
12 detectors (Tseng et al. 2000; Jung et al. 2006; Yuen et al. 2007; Zou et al. 2007; Date et al.  
13  
14 2008; Huang et al. 2008; Wang et al. 2008), capillary electrophoresis (CE) (Cianchino et al.  
15  
16 2008) and IR-LSLS (Lu et al. 2007). None of these methods is ideal as HM often contain  
17  
18 mixture of herbs and other natural products, and the formulas of those products are very  
19  
20 complex. Chromatographic methods do not provide global information as they are mainly  
21  
22 targeted screening methods and thus sometimes too selective. Moreover, false-positive results  
23  
24 might occur with both chromatographic and IR-LSLS methods because it is in fact an  
25  
26 analogue of a known active compound with a very close chemical structure which is detected  
27  
28 instead of the target compound.  
29  
30  
31  
32  
33

34 In this study we show an application of 2D Diffusion Ordered Spectroscopy  $^1\text{H}$  Nuclear  
35  
36 Magnetic Resonance (2D DOSY  $^1\text{H}$  NMR) spectroscopy as an untargeted screening method  
37  
38 for the detection and unequivocal identification of adulterants in “herbal” slimming products.  
39  
40 The main advantage of NMR is to provide global information as it allows for considering the  
41  
42 drug preparation as a whole. Moreover, the method is non-selective and requires no prior  
43  
44 knowledge of the structures of the various components present in the mixture, which is a  
45  
46 major advantage for screening of adulteration.  
47  
48  
49  
50  
51  
52

## 53 **Experimental**

### 54 *Samples*

55  
56 Twenty commercial formulations of HM or DS marketed for weight loss were analyzed.  
57  
58 Formulations **1**, **5**, and **7** were bought in China and formulations **16-18** in Syria. Other  
59  
60 formulations were purchased over the internet. The list of the tablets or capsules analyzed is

1  
2  
3 given in Table 1. All samples, as received, were stored in the dark at ambient temperature and  
4  
5 humidity. They were all analyzed within their expiration dates (when indicated).  
6  
7  
8  
9

### 10 *<sup>1</sup>H and 2D DOSY <sup>1</sup>H NMR*

11 The <sup>1</sup>H NMR experiments were performed on a Bruker Avance 500 spectrometer  
12 equipped with a 5 mm cryoprobe at 298K. Typical acquisition parameters were as follows:  
13 acquisition time 2.72 s, spectral width 6000 Hz, pulse width 3.0 μs (flip angle ≈35°), 32 K  
14 data points, relaxation delay 1 s and number of scans 16. All chemical shifts (δ) were referred  
15 to an internal δ and quantification sodium 2,2,3,3-tetradeutero-3-trimethylsilylpropionate  
16 (TSP) reference. Authentic standards (sibutramine, phenolphthalein, synephrine, vitaberine,  
17 caffeine, carnitine, linoleate, stearate, citrate, vitamin C, dehydroascorbic acid, vitamins B1,  
18 B2, B3, B5, B6, and E, lipoic acid, glucose, fructose and sucrose) were added into the  
19 samples to assign resonances in the <sup>1</sup>H NMR spectra. Moreover, 1D <sup>1</sup>H NMR spectra  
20 extracted from the 2D DOSY <sup>1</sup>H NMR spectrum also helped to substantiate compound  
21 identification.  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37

38 Relaxation delay was lengthened to 3 s and 128 scans were recorded for the quantitative  
39 analysis of sibutramine, phenolphthalein, synephrine, methylsynephrine, caffeine and  
40 vitaberine. The concentrations of adulterant compounds were measured by comparing the  
41 expanded areas of their respective NMR signals with that of the internal standard for  
42 quantification TSP. The areas were determined by manual integration using TopSpin  
43 software. Each data is the mean of at least five integrations. In order to check that the NMR  
44 conditions used allowed an accurate quantitation of adulterants, a longer interval between  
45 pulses (10 s) was used in some experiments. The same concentration values were obtained,  
46 thus demonstrating that a pulse interval of 3 s for a flip angle of 35° is sufficient to record the  
47 spectra under conditions of full T<sub>1</sub> relaxation. Sibutramine was assayed from the singlet at  
48 7.47 ppm, phenolphthalein from the two doublets at 7.17 and 6.84 ppm, synephrine from the  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

1  
2  
3 two doublets at 7.23 and 6.83 ppm, methylsynephrine from the two doublets at 7.20 and 6.83  
4  
5 ppm, caffeine from the singlet at 7.77 ppm in formulation **12** and from the two singlets at 3.93  
6  
7 and 3.50 ppm in formulation **19**, and vitaberine from the triplet at 4.04 ppm. The limit of  
8  
9 quantification of the NMR assay in the recording conditions used in this study is 5  $\mu\text{M}$ .  
10  
11

12  
13 Stimulated echo bipolar gradient pulse experiments were used for 2D DOSY  $^1\text{H}$  NMR  
14  
15 with a pulse field gradient length between 1.4 and 1.8 ms, a delay of 3 ms after each gradient  
16  
17 and a diffusion delay between 50 and 100 ms. Sequence parameters were optimized in order  
18  
19 to have the intensity of the main signals of the spectrum in the aromatic region strongly  
20  
21 decreased (at least divided by 50) at 95% of the full gradient strength. The longitudinal eddy  
22  
23 current delay was 20 ms, spoiler gradients were 1 ms long with a field strength of -7.92 and -  
24  
25 6.09  $\text{G}\cdot\text{cm}^{-1}$  and other acquisition parameters were the same as those reported above for 1D  
26  
27  $^1\text{H}$  NMR except for the relaxation delay fixed to 2 s. Forty experiments were recorded with  
28  
29 gradient intensity linearly sampled from 5 to 95%. The gradient system had been calibrated to  
30  
31 46.25  $\text{G}\cdot\text{cm}^{-1}$  at maximum intensity.  
32  
33  
34  
35

36  
37 All data were processed using the Gifa 5.2 software with the inverse Laplace transform  
38  
39 method using the Maximum Entropy (MaxEnt) algorithm. The processing parameters were  
40  
41 2048 points along the Laplace spectrum diffusion axis and 20000 MaxEnt iterations. The  
42  
43 inverse Laplace transform was computed only on the columns presenting a signal 32 times  
44  
45 greater than the noise level of the experiment. DOSY spectra are presented with chemical  
46  
47 shifts on the horizontal axis and diffusion coefficients expressed in  $\mu\text{m}^2\cdot\text{s}^{-1}$  on the vertical  
48  
49 axis.  
50  
51  
52

53  
54 The 3D DQF-COSY iDOSY experiment was recorded on formulation **7**. In the diffusion  
55  
56 dimension, 28 gradient steps were used with 50 ms for the diffusion delay, 1.9 ms for the  
57  
58 gradient pulse length and 3 ms for the recovery delay. In the COSY dimension,  $8192 \times 104$   
59  
60 data points were used, corresponding to 0.74 s for acquisition time and 8.6 ms for the other



1  
2  
3 dimension, with a spectral window of 12 ppm. The sineshaped gradients for DQF selection, of  
4  
5 4.62 and 9.25 G.cm<sup>-1</sup> strength, were applied for 1 ms. The relaxation delay was 1.3 s and the  
6  
7 number of scans was 8 (half-day experiment). The spectrum was processed using the NPK  
8  
9 software (Tramesel et al. 2007) with Fourier transform in magnitude mode in the COSY  
10  
11 dimension and with the inverse Laplace Transform method (MaxEnt algorithm) in the DOSY  
12  
13 dimension. The processing parameters were 192 points along the diffusion axis and 20000  
14  
15 MaxEnt iterations. The algorithm was computed only on the columns with a signal-to-noise  
16  
17 ratio above 32. The corresponding 2D COSY and DOSY experiments were acquired with the  
18  
19 3D DQF-COSY iDOSY parameters. These experiments were analyzed with the  
20  
21 NMRnotebook package (NMRtec 2007).  
22  
23  
24  
25  
26  
27  
28

### 29 *MS-MS analysis*

30  
31 An Applied System QTRAP triple-quadrupole mass spectrometer, equipped with a  
32  
33 Turbo Ion Spray (TIS) interface and controlled by the Agilent Analyst software (version 1.4),  
34  
35 was used for analysis. The mass spectrometer was operated in positive ionization mode.  
36  
37 Nitrogen served both as auxiliary, collision gas and nebulizer gas. The operating conditions  
38  
39 for TIS interface were as follows: (i) in MS mode: mass range 100–600 u (1 s), step size 0.1  
40  
41 u; Q1 TIS MS spectra were recorded in profile mode, IS 5000 V, DP 40 V; (ii) in MS–MS  
42  
43 mode: precursor mass 280 u; mass range 30–300 u; step size 0.1 u; MS–MS spectra were  
44  
45 recorded in profile mode, IS 5000 V, DP 40 V, CE 20 V. All samples were dissolved in  
46  
47 MeOH and analyzed after direct introduction.  
48  
49  
50  
51  
52  
53  
54

### 55 *Preparation of samples for NMR analysis*

56  
57 The tablets were powdered (or capsule emptied) and dissolved in 5 mL of CD<sub>3</sub>CN:D<sub>2</sub>O  
58  
59 80:20 v/v (CD<sub>3</sub>CN for formulation **8** and D<sub>2</sub>O for formulation **16**) under magnetic stirring  
60

1  
2  
3 during 10 min and then sonicated for 10 min. The suspension was then centrifuged (5 min,  
4  
5 3000 rpm) and the supernatant (550  $\mu\text{L}$ ) analyzed.  
6  
7

8 For the quantitative analysis of sibutramine, phenolphthalein, synephrine,  
9  
10 methylsynephrine, vitaberine and caffeine found in some formulations, the tablet was  
11  
12 powdered (or capsule emptied), and 10% of the powder was dissolved in 10 mL of methanol  
13  
14 under magnetic stirring during 15 min and then sonicated for 10 min. An aliquot of 1 mL was  
15  
16 evaporated to dryness and the residue dissolved in 1 mL of  $\text{MeOH-d}_4$ . TSP was added at a  
17  
18 final concentration of 0.2 mM before the NMR analysis. The experiments were done in  
19  
20  
21  
22 triplicate.  
23  
24  
25  
26  
27

## 28 Results

### 29 *Conventional $^1\text{H}$ NMR and 2D DOSY $^1\text{H}$ NMR analyses*

30  
31  
32 All formulations were analyzed with 2D DOSY  $^1\text{H}$  NMR. 2D DOSY spectra of  
33  
34 formulations **3**, **12** and **19** along with their corresponding 1D spectrum are presented in Figure  
35  
36 1. All the peaks of a same compound are lined up. The peaks at 3.62 and 1.99 ppm correspond  
37  
38 to the signals of residual HOD in  $\text{D}_2\text{O}$  and  $\text{CHD}_2\text{CN}$  in acetonitrile- $\text{d}_3$ , respectively. As shown  
39  
40 in Figure 1, the separation according to diffusion coefficients ( $D$ ) in the diffusion dimension  
41  
42 allows an easier identification of the components of a mixture. Indeed, in formulation **3**  
43  
44 (Figure 1A), the active pharmaceutical ingredient (API) adulterant is sibutramine ( $D = 1107 \pm$   
45  
46  $9 \mu\text{m}^2 \cdot \text{s}^{-1}$ ) citrate ( $D = 959 \mu\text{m}^2 \cdot \text{s}^{-1}$ ) and low amounts of natural compounds including glucose,  
47  
48 sucrose, and fatty acids ( $D = 931 \pm 29$ ,  $1006 \pm 29$ , and  $872 \pm 29$ , respectively) are also  
49  
50 detected. In formulation **12** (Figure 1B), the main signals are those of caffeine ( $D = 1156 \pm$   
51  
52  $39 \mu\text{m}^2 \cdot \text{s}^{-1}$ ) and synephrine ( $D = 734 \pm 20 \mu\text{m}^2 \cdot \text{s}^{-1}$ ), whereas formulation **19** (Figure 1C)  
53  
54 contains caffeine, methylsynephrine, vitaberine (thiamine-O-isobutyryl disulphide) and  
55  
56  
57  
58  
59  
60

1  
2  
3 stearate with diffusion coefficients of  $1288 \pm 93$ ,  $851 \pm 41$ ,  $624 \pm 9$  and  $918 \mu\text{m}^2.\text{s}^{-1}$ ,  
4  
5 respectively.  
6

7  
8 All the ingredients detected in the herbal drugs studied are reported in Table 2, which  
9  
10 shows the comparison between the indications provided by the manufacturer and the  
11  
12 compounds found in each formulation. Several unexpected actives, sibutramine (formulations  
13  
14 **1-5, 7-11, 13-15**), phenolphthalein (formulations **1, 5, 7, 9, 15**) synephrine and caffeine  
15  
16 (formulation **12**), were found. The unambiguous identification of the components in the  
17  
18 herbal formulations analyzed was achieved by comparison of 1D  $^1\text{H}$  NMR data of standards,  
19  
20 addition of authentic standards, and comparison of 1D NMR spectra extracted from the 2D  
21  
22 DOSY  $^1\text{H}$  NMR spectrum with those of authentic standards (Figure 1D).  
23  
24  
25

26  
27 The NMR spectral data of sibutramine measured in  $\text{CD}_3\text{CN}:\text{D}_2\text{O}$  (80:20) are reported in  
28  
29 Table 3. The  $^1\text{H}$  NMR resonances were assigned by 2D NMR (gCOSY, gHSQC, gHMBC,  
30  
31 gNOESY) and 1D selective NOE experiments. The chemical shifts and coupling constants of  
32  
33 all the other ingredients are reported in Table 4.  
34  
35

36  
37 Even if sensitivity is not an issue with respect to adulteration, we determined that in the  
38  
39 experimental conditions used in this study (1h of recording), 2D DOSY  $^1\text{H}$  NMR allows an  
40  
41 accurate determination of diffusion coefficients for compounds at a concentration of  $50 \mu\text{M}$ .  
42  
43 The limit of detection corresponds to  $10 \mu\text{M}$  with only  $\text{CH}_2$  and  $\text{CH}_3$  signals being detected.  
44  
45

### 46 47 48 ***3D DOSY-COSY $^1\text{H}$ NMR analysis*** 49

50  
51 Figure 2 illustrates the different NMR spectra obtained in the 3D DOSY-COSY  $^1\text{H}$   
52  
53 NMR experiment applied to formulation **7**. First, the classical 2D DOSY (Figure 2A) and  
54  
55 COSY-DQF (Figure 2B) spectra are shown. In the COSY spectrum, diagonal signals and off-  
56  
57 diagonal cross peaks of sibutramine, phenolphthalein, glucose and sucrose are observed.  
58  
59 Other spectra (2C and 2D) are those of the projections from the 3D DOSY-COSY  
60  
experiment. The main interest of this 3D experiment is to extract the COSY spectrum of each

1  
2  
3 component of the mixture from a selected line in the DOSY spectrum. For example, Figure  
4  
5  
6 2C shows the COSY projection of phenolphthalein at  $D = 990 \mu\text{m}^2.\text{s}^{-1}$ ; typical cross peaks of  
7  
8 aromatic signals are detected for this compound while the on-diagonal signal from aromatic  
9  
10 protons of sibutramine has disappeared. The COSY projection corresponding to  $D = 711$   
11  
12  $\mu\text{m}^2.\text{s}^{-1}$  (Figure 2D) shows exclusively the signals of sucrose without any signal from  
13  
14 phenolphthalein or anomeric protons from  $\alpha$ - or  $\beta$ -glucose. These spectra highlight the interest  
15  
16 of 3D experiment as virtual separation provided by DOSY acquisition can lead to structural  
17  
18 determination by extraction of COSY spectra.  
19  
20  
21  
22  
23  
24

### 25 *Quantitative analysis*

26  
27 The contents of sibutramine and phenolphthalein in the herbal drugs analyzed were  
28  
29 measured by NMR (Table 5). Eight formulations (**2, 3, 4, 8, 10, 11, 13, 14**) contain  
30  
31 sibutramine alone at doses ranging from 4.4 to 30.5 mg/capsule. Five formulations (**1, 5, 7, 9,**  
32  
33 **15**) contain sibutramine (from 5.0 to 19.6 mg/capsule or tablet) and phenolphthalein (from 4.4  
34  
35 to 66.1 mg/capsule). Formulation **12** contains  $19.5 \pm 0.5$  and  $10.0 \pm 0.5$  mg/capsule of  
36  
37 synephrine and caffeine, respectively. Formulation **19** contains  $38.3 \pm 0.9$ ,  $184.9 \pm 5.6$  and  
38  
39  $75.4 \pm 1.6$  mg/capsule of methylsynephrine, caffeine and vitaberine, respectively.  
40  
41  
42  
43  
44

### 45 *Mass spectrometry*

46  
47 The aim of mass spectrometry experiments was to cross-validate NMR data. MS/MS  
48  
49 mass spectrometry with ESI ionization was carried out in order to confirm the presence of  
50  
51 sibutramine and to investigate the presence of its previously described mono- or di-  
52  
53 desmethylated analogs (Wang et al. 2008). Molecular  $[\text{M}+\text{H}]^+$  ions corresponding to  
54  
55 sibutramine, N-mono-desmethylsibutramine and N-di-desmethylsibutramine were searched at  
56  
57  $m/z$  280, 266 and 252, respectively (Wang et al. 2008). Only sibutramine was found. MS/MS  
58  
59 was then carried out on the  $m/z$  280 ion giving characteristic fragments at  $m/z$  153, 139, and  
60

1  
2  
3 125. The presence of sibutramine was thus confirmed by MS/MS in thirteen formulations (**1-**  
4 **5, 7-11, and 13-15**). MS was also used to confirm the presence of methylsynephrine,  
5  
6 vitaberine and caffeine ( $[MH]^+$  ions at  $m/z$  182, 703, and 195 respectively) in formulation **19**  
7  
8 and synephrine ( $[MH]^+$  ion at  $m/z$  168) in formulation **12**.  
9  
10  
11

## 12 13 14 15 **Discussion**

16  
17 To date, identification of synthetic drugs adulterating HM to inhibit appetite and achieve  
18 weight loss using NMR and more specifically 2D DOSY  $^1H$  NMR has not been reported. The  
19 objective of this study was to apply this method to analyze the content of 20 different HM or  
20 DS.  
21  
22  
23  
24  
25

26  
27 Chromatographic methods are preferred by most analysts for mixture analysis as they  
28 are very common, easy to handle, quantitative, sensitive, relatively cheap and do not require  
29 highly trained personnel. Nevertheless, NMR has also several advantages; it is non-selective,  
30 quantitative, highly powerful for structural elucidation and it requires no prior knowledge of  
31 the structures of the various components present in a mixture. 2D DOSY  $^1H$  NMR allows the  
32 virtual separation of several components of a mixture in a single run based on the difference  
33 in their translational self-diffusion coefficients (Figure 1). Moreover, DOSY experiments do  
34 not need complicated setup procedures or separation steps and the method can be used  
35 routinely. This technique has been used successfully for analyzing several kinds of counterfeit  
36 drugs (Trefi et al. 2008; Nyadong et al. 2009). Table 6 shows a comparison on information  
37 obtained with various analytical methods on slimming herbal drug composition and highlights  
38 the interest of DOSY NMR for a global analysis of these formulations.  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53

54  
55 Among the 20 formulations analyzed, 2 were strictly herbal (formulations **17** and **18**),  
56 and 4 had a composition corresponding to declared ingredients on the packaging or the leaflet  
57 (formulations **6, 16, 19, 20**). The others were all adulterated. Thirteen formulations (**1-5, 7-11,**  
58  
59  
60

1  
2  
3 **13-15**) contained sibutramine alone or in combination with phenolphthalein (**1, 5, 7, 9,** and  
4  
5 **15**). The two metabolites of sibutramine, N-mono- and N-di-desmethylsibutramine,  
6  
7 previously described by some authors (Blachut et al. 2007; Lai et al. 2007; Yuen et al. 2007;  
8  
9 Huang et al. 2008) as possible adulterants in slimming dietary supplements were not detected  
10  
11 in our samples neither with NMR nor with MS and MS/MS analyses. The amount of  
12  
13 sibutramine found in adulterated formulations ranged from 4.4 (formulation **2**) to 30.5  
14  
15 mg/capsule (formulation **11**). The recommended daily dose is 5 to 15 mg, 15 mg being the  
16  
17 maximum daily dose (de Simone and D'Addeo 2008). Three formulations (**3, 5** and **11**) were  
18  
19 thus overdosed, which poses a serious health risk to consumers and particularly a  
20  
21 cardiovascular risk as an increase in systolic blood pressure has been noticed in patients  
22  
23 receiving sibutramine at the non recommended doses of 20 or 30 mg (Narkiewicz 2002).  
24  
25 Several cases of mild to severe poisoning due to herbal slimming medicines adulterated with  
26  
27 sibutramine (Lida (Jung et al. 2006; Müller et al. 2009), Ever Youth and unknown (Yuen et  
28  
29 al. 2007), Meizitanc (Sein Anand and Chodorowski, 2007)) have been recently reported. It is  
30  
31 interesting to point out that during the reviewing process of this article, the European  
32  
33 Medicines Agency's Committee for Medicinal Products for Human Use has concluded that  
34  
35 the benefits of sibutramine do not outweigh its risks, and that all marketing authorizations for  
36  
37 medicines containing sibutramine should be suspended throughout Europe  
38  
39 (<http://www.ema.europa.eu/pdfs/human/referral/sibutramine/3940810en.pdf>).  
40  
41  
42  
43  
44  
45  
46  
47

48  
49 Other actives found may also cause serious adverse effects. Phenolphthalein is a laxative  
50  
51 that was withdrawn as a medicine several years ago after concerns about carcinogenicity (No  
52  
53 authors listed 1999). Moreover, interaction profiles are unknown for the combination  
54  
55 sibutramine/phenolphthalein. After the ban on ephedrines in 2004, manufacturers changed to  
56  
57 using the weaker stimulant synephrine that has purported thermogenic and lipolytic properties  
58  
59 due to its  $\alpha$ -adrenergic agonist property (Haaz et al. 2006). The safety of synephrine is  
60

1  
2  
3 controversial but it could pose cardiovascular health risks (Haaz et al. 2006), especially in  
4 combination with caffeine (Haller et al. 2005). Formulation **12**, claimed as purely herbal,  
5  
6 contains both compounds and formulation **19** contains methylsynephrine (para-  
7  
8 hydroxyephedrine) in combination with a high dose of caffeine, a potentially hazardous  
9  
10 combination.  
11  
12  
13  
14  
15  
16  
17

## 18 **Conclusion**

19  
20 This study presents a new application of 2D DOSY  $^1\text{H}$  NMR for the analysis of HM and  
21  
22 DS, which are undeniable complex mixtures. Among the twenty formulations marketed for  
23  
24 weight loss studied thirteen were adulterated with sibutramine alone or with a combination of  
25  
26 sibutramine and phenolphthalein. Adulteration of “natural herbal medicines” with undeclared  
27  
28 synthetic drugs is a common and dangerous phenomenon and all the analytical methods that  
29  
30 ensure the quality and safety of these products have thus to be developed. In this context, 2D  
31  
32 DOSY  $^1\text{H}$  NMR spectroscopy is a powerful method for providing a multivariate fingerprint of  
33  
34 a very complex mixture especially in situations where the identity of the components is not  
35  
36 known beforehand as it permits to consider the drug preparation as a whole. The technique  
37  
38 should be now considered as a useful and complementary tool among a standard 2D NMR  
39  
40 analytical package. Moreover, its evolution towards 3D DOSY-COSY experiments noticeably  
41  
42 increases its interest as structural data are easily obtained.  
43  
44  
45  
46  
47  
48  
49

## 50 **Acknowledgements**

51  
52 The authors wish to thank Marie-José Parent and Dr Saleh Trefi for helpful discussion  
53  
54 on herbal drug adulteration and Catherine Claparols for mass spectrometry experiments.  
55  
56  
57  
58  
59  
60

## References

- Blachut D, Siwinska-Ziolkowska A, Szukalski B, Wojtasiewicz K, Czarnocki Z, Kobylecka A, Bykas-Strekowska M. 2007. Identification of N-desmethylsibutramine as a new ingredient in Chinese herbal dietary supplements. *Probl Forensic Sci.* 70:225-235.
- Bogusz MJ, Hassan H, Al-Enazi E, Ibrahim Z, Al-Tufail M. 2006 Application of LC-ESI-MS-MS for detection of synthetic adulterants in herbal remedies. *J Pharm Biomed Anal.* 41:554-564.
- Chen Y, Zhao L, Lu F, Yu Y, Chai Y, Wu Y. 2009. Determination of synthetic drugs used to adulterate botanical dietary supplements using QTRAP LC-MS/MS. *Food Add Cont.* 26:595-603.
- Cianchino V, Acosta G, Ortega C, Martinez LD, Gomez MR. 2008. Analysis of potential adulteration in herbal medicines and dietary supplements for the weight control by capillary electrophoresis. *Food Chem.* 108:1075-1081.
- Date H, Toyota A, Terauchi M, Sugimura M, Matsuo T, Mochiike C. 2008. Rapid determination of medical components found in the health food for weight loss by liquid chromatography/tandem mass spectrometry (LC/MS/MS). *Yakugaku Zasshi.* 128:811-817.
- de Simone G, D'Addeo G. 2008. Sibutramine: balancing weight loss benefit and possible cardiovascular risk. *Nutr Metab Cardiovasc Dis.* 18:337-341.
- Haaz S, Fontaine KR, Cutter G, Limdi N, Perumean-Chaney S, Allison DB. 2006. Citrus aurantium and synephrine alkaloids in the treatment of overweight and obesity: an update. *Obes Rev.* 7:79-88.
- Haller CA, Benowitz NL, Jacob P. 2005. Hemodynamic effects of ephedra-free weight-loss supplements in humans. *Am J Med.* 118:998-1003.
- Huang Z, Xiao S, Luo D, Chen B, Yao S. 2008. Simultaneous determination of sibutramine and N-di-desmethylsibutramine in dietary supplements for weight control by HPLC-ESI-MS. *J Chromatogr Sci.* 46:707-711.
- Jung J, Hermanns-Clausen M, Weinmann W. 2006. Anorectic sibutramine detected in a Chinese herbal drug for weight loss. *Forensic Sci Int.* 161:221-222.
- Lai KC, Liu YC, Tseng MC, Lin YL, Lin JH 2007 Isolation and identification of a sibutramine analogue in a healthy food for weight loss. *J Food Drug Anal.* 15:20-24.
- Lau AJ, Holmes MJ, Woo SO, Koh HL. 2003. Analysis of adulterants in a traditional herbal medicinal product using liquid chromatography-mass spectrometry-mass spectrometry. *J Pharm Biomed Anal.* 31:401-406.
- Liang Q, Qu J, Luo G, Wang Y. 2006. Rapid and reliable determination of illegal adulterant in herbal medicines and dietary supplements by LC/MS/MS. *J Pharm Biomed Anal.* 40:305-311.
- Liu SY, Woo SO, Koh HL. 2001. HPLC and GC-MS screening of Chinese proprietary medicine for undeclared therapeutic substances. *J Pharm Biomed Anal.* 24:983-992.



1  
2  
3 Lu F, Li S, Le J, Chen G, Cao Y, Qi Y, Chai Y, Wu Y. 2007. A new method for testing  
4 synthetic drugs adulterated in herbal medicines based on infrared spectroscopy. *Anal Chim*  
5 *Acta.* 589:200-207.  
6

7  
8 Müller D, Weinmann W, Hermanns-Clausen M. 2009. Chinese slimming capsules containing  
9 sibutramine sold over the Internet: a case series. *Dtsch Arztebl Int.* 106:218-222.  
10

11 Narkiewicz K. 2002. Sibutramine and its cardiovascular profile. *Int J Obes.* 26:S38-S41.  
12

13 Nyadong L, Harris GA, Balayssac S, Galhena AS, Malet-Martino M, Martino R, Parry RM,  
14 Wang MD, Fernandez FM, Gilard V. 2009. Combining two-dimensional diffusion-ordered  
15 nuclear magnetic resonance spectroscopy, imaging desorption electrospray ionization mass  
16 spectrometry and direct analysis in real time mass spectrometry for the integral investigation  
17 of counterfeit pharmaceuticals. *Anal Chem.* 81:4803-4812.  
18

19  
20 NMRtec, Nmrnotebook software. Available from: <<http://www.NMRnotebook.com>>  
21

22  
23 No authors. 1999. Laxative drug products for over-the-counter human use. Food and Drug  
24 Administration, HHS. Final rule. *Fed Regist.* 64:4535-4540.  
25

26  
27 Sein Anand J, Chodorowski Z. 2007. Side effects after the usage of Chinese dieting product  
28 Meizitanc. *Przegl Lek.* 64:346-347.  
29

30 Tramesel D, Catherinot V, Delsuc MA. 2007. Modeling of NMR processing, toward efficient  
31 unattended processing of NMR experiments. *J Magn Reson* 188:56-67.  
32

33 Trefi S, Routaboul C, Hamieh S, Gilard V, Malet-Martino M, Martino R. 2008. Analysis of  
34 illegally manufactured formulations of tadalafil (Cialis®) by <sup>1</sup>H NMR, 2D DOSY <sup>1</sup>H NMR  
35 and Raman spectroscopy. *J Pharm Biomed Anal.* 47:103-113.  
36

37  
38 Tseng MC, Tsai MJ, Lin JH, Wen KC. 2000. GC/MS analysis on anorectics adulterated in  
39 traditional Chinese medicines. *J Food Drug Anal.* 8:315-330.  
40

41  
42 Wang J, Chen B, Yao S. 2008. Analysis of six synthetic adulterants in herbal weight-reducing  
43 dietary supplements by LC electrospray ionization-MS. *Food Addit Contam.* 25:822-830.  
44

45 Yuen YP, Lai CK, Poon WT, Ng SW, Chan AYW, Mak TWL. 2007. Adulteration of over-  
46 the-counter slimming products with pharmaceutical analogue-an emerging threat. *Hong Kong*  
47 *Med J.* 13:216-220.  
48

49  
50 Zhu X, Zhang Z, Lu F, Wu Y, Qi Y. 2009. Modified local straight-line screening to detect  
51 synthetic drugs in adulterated herbal medicines. *Appl Spectrosc.* 63:471-476  
52

53 Zou P, Oh SS-Y, Kiang K-H, Low M-Y, Bloodworth BC. 2007. Detection of sibutramine, its  
54 two metabolites and one analogue in a herbal product for weight loss by liquid  
55 chromatography triple quadrupole mass spectrometry and time-of-flight mass spectrometry.  
56 *Rapid Commun Mass Spectrom.* 21:614-618.  
57  
58  
59  
60

### Captions for figures

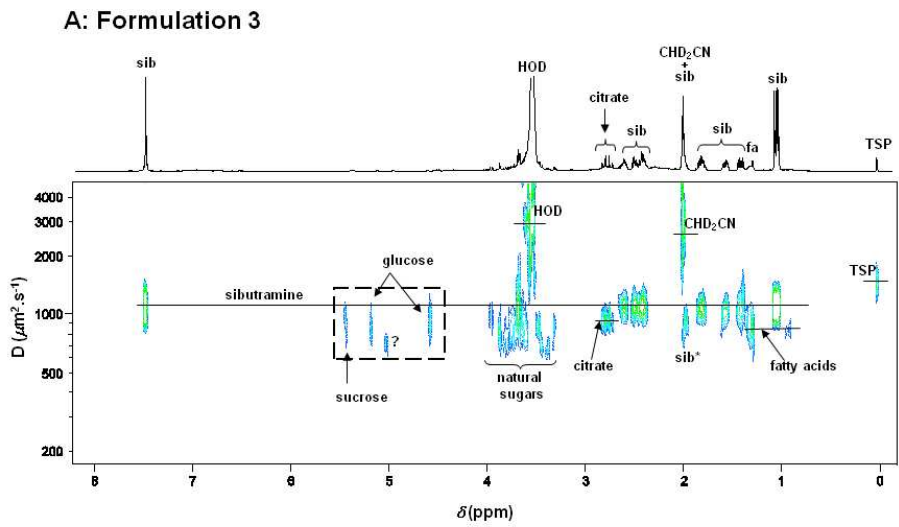
**Figure 1. 2D DOSY  $^1\text{H}$  NMR spectra recorded in  $\text{CD}_3\text{CN}:\text{D}_2\text{O}$  (80/20) of formulations 3 (A), 12 (B) and 19 (C). (D) Comparison of 1D  $^1\text{H}$  NMR spectra of caffeine (D1) and vitaberine (D2) extracted from the 2D DOSY  $^1\text{H}$  NMR spectrum of formulation 19 with those of authentic standards.**

sib, sibutramine; caf, caffeine; syn, synephrine; msyn, methylsynephrine; vita: vitaberine; fa, fatty acids; st, stearate; TSP, sodium 2,2,3,3-tetradeutero-3-trimethylsilylpropionate (internal standard); ?, unknown; sib\* means that the diffusion coefficient of this sibutramine signal is lower due to its superimposition with  $\text{CHD}_2\text{CN}$ , which affects DOSY processing. A deeper section of some signals is shown in box with dotted lines.

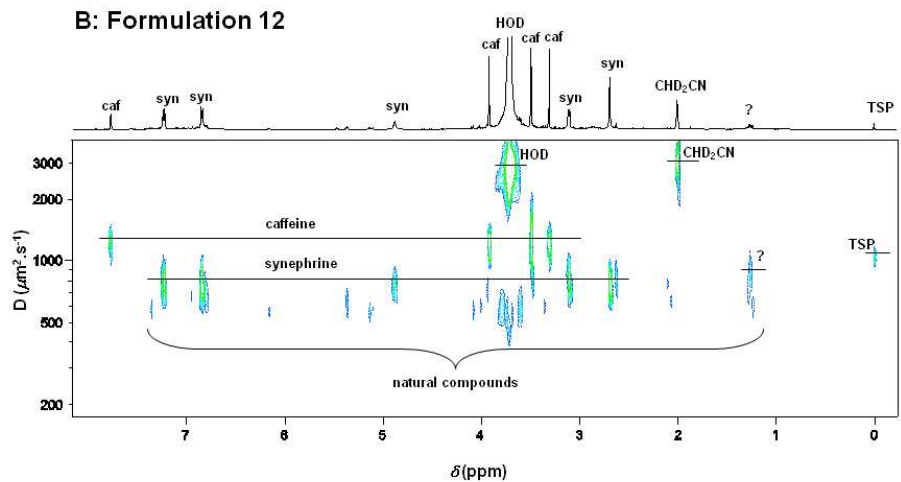
**Figure 2. NMR spectra of formulation 7 in  $\text{CD}_3\text{CN}:\text{D}_2\text{O}$  (80/20). (A) 2D DOSY  $^1\text{H}$  spectrum; (B) COSY-DQF spectrum; COSY extractions from 3D DOSY-COSY experiment at (C)  $D = 990 \mu\text{m}^2 \cdot \text{s}^{-1}$ , (D)  $D = 711 \mu\text{m}^2 \cdot \text{s}^{-1}$ .**

sib, sibutramine; phth, phenolphthalein; suc, sucrose; gluc, glucose; st, stearate; S, satellite signals from  $\text{CHD}_2\text{CN}$ ; TSP, sodium 2,2,3,3-tetradeutero-3-trimethylsilylpropionate (internal standard); sib\* means that the diffusion coefficient of this sibutramine signal is lower due to its superimposition with  $\text{CHD}_2\text{CN}$ , which affects DOSY processing.

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60



254x190mm (96 x 96 DPI)

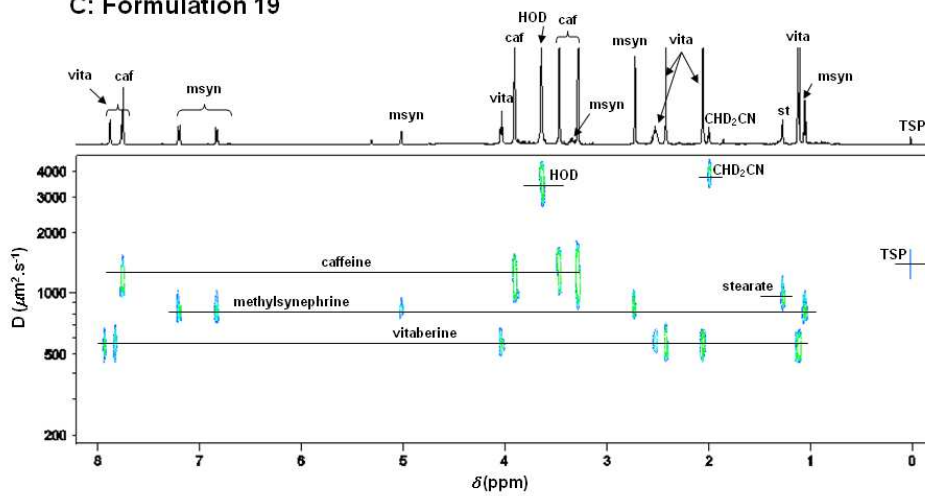


254x190mm (96 x 96 DPI)

View Only

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

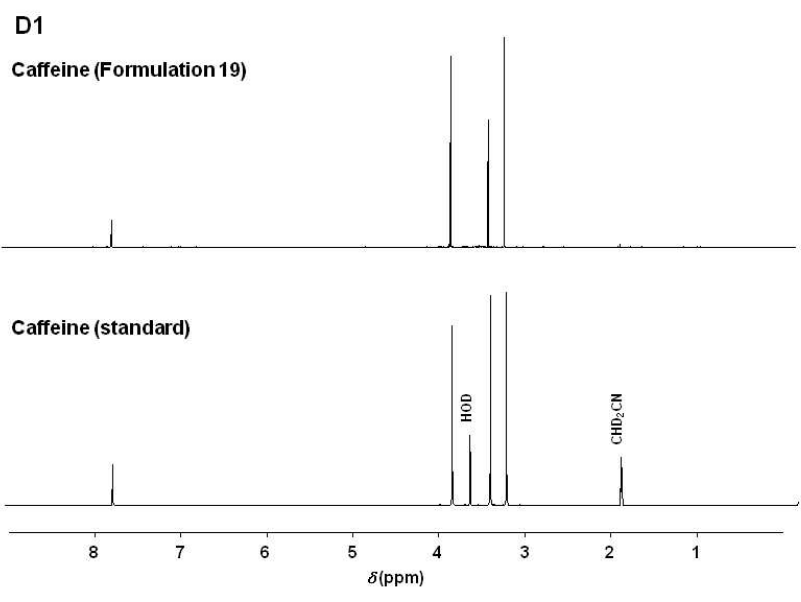
C: Formulation 19



254x190mm (96 x 96 DPI)

View Only

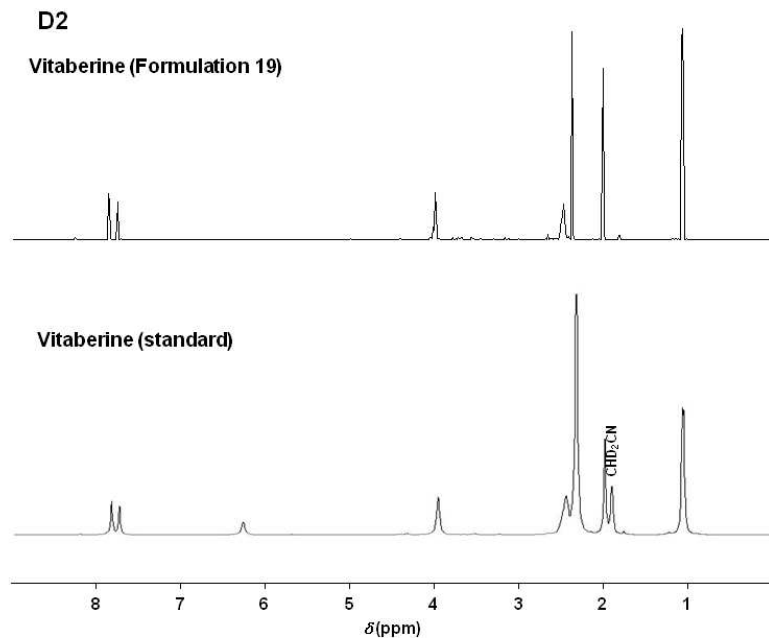
1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60



254x190mm (96 x 96 DPI)

View Only

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60



254x190mm (96 x 96 DPI)

View Only

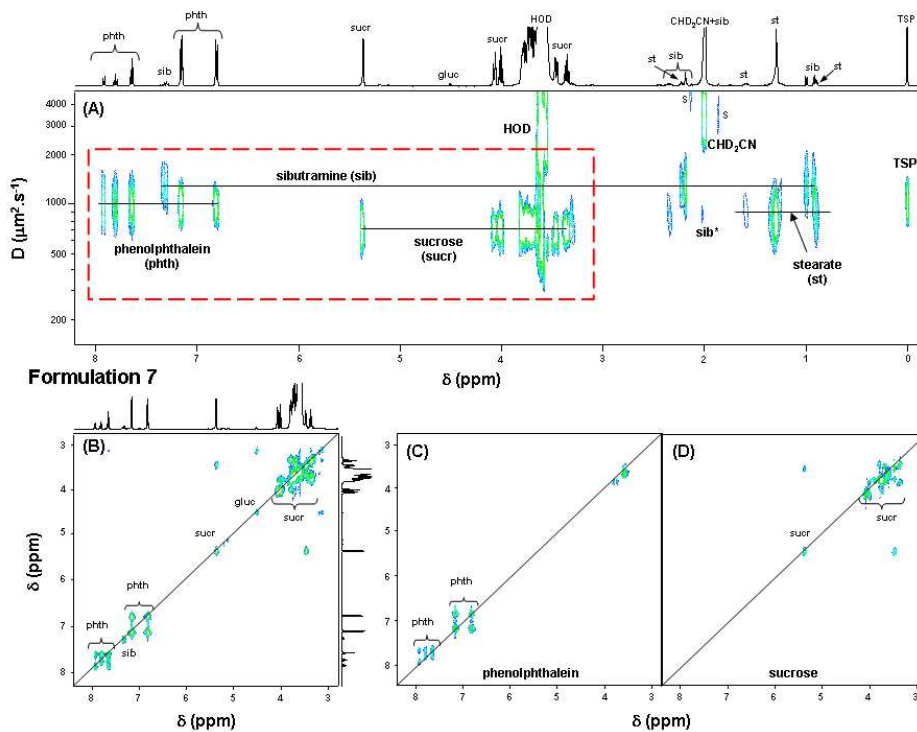


Fig 2  
254x190mm (96 x 96 DPI)



**Table 1.** Herbal commercial formulations analyzed in this study.

	<b>Formulation name</b>	<b>Batch number</b>	<b>Expiration date</b>	<b>Manufacturer name</b>	<b>Country of manufacturing</b>	<b>Product form</b>	<b>Color</b>
<b>1</b>	Perfect slim cherry	2007070183	07/2009	Chun Tian	Japan	Capsule	Pink
<b>2</b>	9 rapide 9	Q/CL5-2004n0487	05/2009	Zhengda health food	China	Capsule	Red
<b>3</b>	Unknown <sup>a</sup>	Unknown	Unknown	Unknown	China	Capsule	Red and white
<b>4</b>	Unknown <sup>a</sup>	Unknown	Unknown	Menova	China	Capsule	Blue
<b>5</b>	Perfect slim grape	2007080601	08/2009	Chun Tian	Japan	Capsule	Green and white
<b>6</b>	Honey life	20080302	03/2010	B&H (USA)	China	Capsule	Mauve and white
<b>7</b>	Cider vinegar dieting	20080216	02/2010	Qinghai Oifield	China	Tablet	Yellow (with green spots)
<b>8</b>	Botanical slimming soft gel	20081006	10/2010	Meizitang	China	Capsule with gel	Green
<b>9</b>	Botanical slimming Herbs	0991-8280809	08/2010	Meizitang	China	Capsule	Orange and brown
<b>10</b>	2 Day Diet Japan Lingzhi slimming formula	Unknown	12/2012	Unknown	Japan	Capsule	Purple
<b>11</b>	Lipro diet pills	20081020	10/2010	China Karyhen International Co.	China	Capsule	Green

12	Lida	20080924	09/2010	Kunming Dali Industry and Trade Co.	China	Capsule	Green
13	Onting quianweisu slimming herbs <sup>a</sup>	Unknown	03/2010	Menova	China	Capsule	Blue
14	Meekeyes <sup>a</sup>	95882108	Unknown	Marlian	China	Capsule	Green and white
15	Perfect slim <sup>a</sup>	Unknown	Unknown	Unknown	China	Capsule	Pink
16	Garslim	22	05/2011	Balsam	Syria	Tablet	Beige
17	Alattar psyllium	4	06/2009	Alattar Medical Co.	Syria	Capsule	Green
18	Alattar slimming mixture	255161M2	06/2009	Alattar Medical Co.	Syria	Powder	-
19	Hyper drive 3.0 +	110802	10/2013	ALR industries	USA	Capsule	Red
20	Elan-Sil	9.026.6	01/2001	Vitaline Ltd	UK	Capsule	White

<sup>a</sup>This formulation has been seized by French Customs.

Table 2. Comparison of formulation composition with indications provided by the manufacturer.

	<b>Formulation name</b>	<b>Indications provided by the manufacturer</b>	<b>Compounds found</b>	
<b>1</b>	Perfect slim cherry	Only natural compounds	Sibutramine Phenolphthaleine Vitamin C <sup>a</sup> Very low amounts of natural compounds including glucose, fructose, fatty acids	Identical to formulation <b>15</b>
<b>2</b>	9 rapide 9	Only natural compounds	Sibutramine Very low amounts of natural compounds including fatty acids	
<b>3</b>	Unknown	No indication <sup>b</sup>	Sibutramine citrate Low amounts of natural compounds including glucose, fructose, fatty acids	
<b>4</b>	Unknown	No indication <sup>b</sup>	Sibutramine Low amounts of natural compounds including glucose, fructose, fatty acids	Identical to formulation <b>13</b>
<b>5</b>	Perfect slim grape	Only natural compounds	Sibutramine Phenolphthaleine Very low amounts of natural compounds including glucose, fructose, fatty acids	Very similar to formulation 1 except that it contains no vitamin C
<b>6</b>	Honey life	L-carnitine L-tartrate Natural compounds (Konjac flour, Cassia seed)	Carnitine tartrate Low amounts of natural compounds including sugars, fatty acids and vitamin B3	
<b>7</b>	Cider vinegar dieting	Carnitine Flavone glycoside Oligosaccharide	Sibutramine Phenolphthaleine Stearate Sucrose Very low amounts of natural sugars	No carnitine

8	Botanical slimming soft gel	Only natural compounds	Sibutramine Soybean oil	Soybean oil is used to prepare the gel
9	Botanical slimming herbs	Only natural compounds	Sibutramine Phenolphthaleine Very low amounts of natural compounds including glucose, sucrose, fatty acids	
10	2 Day Diet Japan Lingzhi slimming formula	Only natural compounds	Sibutramine Very low amounts of natural compounds including glucose, fatty acids	
11	Lipro diet pills	Only natural compounds (Chinese herbs)	Sibutramine Stearate Very low amounts of natural sugars including glucose	
12	Lida	Only natural compounds (Yunnan herbs)	Caffeine Synephrine Low amount of natural compounds	
13	Onting quianweisu slimming herbs	No indication <sup>b</sup>	Sibutramine Low amounts of natural compounds including glucose, fructose, fatty acids	Identical to formulation 4
14	Meekeyes	No indication <sup>b</sup>	Sibutramine Sucrose Fatty acid High amount of sinapine and very low amounts of other natural compounds	
15	Perfect slim	No indication <sup>b</sup>	Sibutramine Phenolphthaleine Vitamin C <sup>a</sup> Very low amounts of natural compounds including glucose, fructose, fatty acids	Identical to formulation 1
16	Garslim	Fruit extracts with 50% hydroxycitric acid and citric acid	Hydroxycitric acid Citric acid	

17	Alattar psyllium	Mucilages, fatty oil, iridoide monoterpenes	Fatty acids including stearic and linoleic acids	
18	Alattar slimming mixture	Only natural compounds	Only natural compounds including caffeine, polyphenols, sugars, fatty acids	
19	Hyper drive 3.0	Methylsynephrine Xanthines Vitaberine Lipoic acid Tyrosine ethyl ester Propionylcarnitine ethyl ester <sup>c</sup> Natural compounds	Methylsynephrine Caffeine Vitaberine Stearate Very low amounts of natural compounds	The amounts of lipoic acid and tyrosine ethyl ester are probably too low to be detected. Both compounds were undetected with <sup>1</sup> H NMR or MS.
20	Elan-Sil	L-carnitine L-tartrate Vitamin C Vitamin E acetate Vitamin B1 Vitamin B2 Vitamin B3 Vitamin B5 (Ca pantothenate) Vitamin B6 Vitamin B7 Vitamin B9 Vitamin B12 Mg salts of fatty acids	Carnitine Vitamin C <sup>a</sup> Vitamin E acetate Vitamin B1 Vitamin B3 Vitamin B5 Vitamin B6 Stearate	Tartrate was not detected. The amounts of vitamins B2, B7, B9 and B12 are too low to be detected.

<sup>a</sup> Vitamin C is slightly unstable in the extraction conditions used. It was thus detected as a mixture of ascorbic and dehydroascorbic acids.

<sup>b</sup> This formulation was provided by French Customs with no packaging.

<sup>c</sup> Incorrect chemical nomenclature as propionylcarnitine is the propionic ester of carnitine.

Table 3. NMR characteristics of sibutramine detected in the herbal formulations analyzed. Spectra were recorded in CD<sub>3</sub>CN:D<sub>2</sub>O (80:20).

Sibutramine			
Atom number	$\delta$ <sup>1</sup> H (ppm) <sup>a</sup>	Multiplicity <sup>b</sup> (J in Hz)	$\delta$ <sup>13</sup> C (ppm)
1			49.2
2 or 4	2.60 (1H syn aromatic ring) 2.49 (1H anti aromatic ring)	m m	32.5
3	1.98 (1H) 1.78 (1H)	m m	15.5
4 or 2	2.37 (1H syn aromatic ring) 2.41 (1H anti aromatic ring)	m m	33.7
5	-	-	140.2
6,10	7.47	s	128.7
7,9			129.9
8			132.9
11	3.67	t (6.3)	71.7
12 or 13	2.19	broad s	39.6 <sup>c</sup>
13 or 12	2.81	broad s	47.0 <sup>c</sup>
14	1.53 (1H) 1.39 (1H)	m m	33.5
15	1.78	m	25.4
16 or 17	1.01	d (6.5)	22.0
17 or 16	1.04	d (6.5)	21.2

<sup>a</sup> The value given is the chemical shift of the centre of the signal.

<sup>b</sup> s: singlet; d: doublet; t: triplet; m: multiplet.

<sup>c</sup> The chemical shift of this carbon was measured in a spectrum recorded in CD<sub>3</sub>CN.

Table 4. <sup>1</sup>H NMR characteristics of the main signals of ingredients found in herbal formulations (except sibutramine given in Table 3).

Compound	<sup>1</sup> H NMR δ (ppm) <sup>a</sup> and multiplicity <sup>b</sup> (J, Hz)
phenolphthalein	7.93 d (7.5; H8), 7.79 t (7.5; H6), 7.63 t (7.0; H7), 7.63 d (7.5; H6), 7.17 d (8.5; 4 H11), 6.84 d (8.5; 4 H12)
synephrine	7.23 d (8.5; Ar), 6.83 d (8.5; Ar), 4.70 dd (4.3, 8.8; <u>CHOH</u> ), 2.70 ABd system (12.3, 8.8, 4.3; CH <sub>2</sub> ), 2.36 s (CH <sub>3</sub> )
methylsynephrine <sup>c</sup>	7.20 d (8.5; Ar), 6.83 d (8.5; Ar), 5.00 d (3.1; <u>CHOH</u> ), 3.34 qd (3.1, 6.7; <u>CHNH</u> ), 2.71 s (CH <sub>3</sub> NH), 1.04 d (6.7; CH <sub>3</sub> CH)
vitaberine (sulbutiamine)	7.86 s (H pyrim), 7.77 s (CHO), 4.45 centre of a very broad s (CH <sub>2</sub> N), 4.04 t (6.3; CH <sub>2</sub> O), 2.55 sept (7.0; CH(CH <sub>3</sub> ) <sub>2</sub> ), 2.52 broad t (6.3; CH <sub>2</sub> CH <sub>2</sub> O), 2.39 s (CH <sub>3</sub> pyrim), 2.05 s (CH <sub>3</sub> C=C-), 1.12 d (7.0; CH(CH <sub>3</sub> ) <sub>2</sub> )
riboflavine	8.00 s (Ar), 7.99 s (Ar), 4.93 m (CH <sub>2</sub> N), 4.37 m ( <u>CHOH</u> ), 3.87 m ( <u>CHOH</u> ), 3.79 m ( <u>CH<sub>2</sub>OH</u> ), 3.66 m ( <u>CHOH</u> ), 2.58 s (CH <sub>3</sub> -Ar), 2.48 s (CH <sub>3</sub> -Ar)
caffeine	7.77 s (H), 3.93 s (CH <sub>3</sub> ), 3.50 s (CH <sub>3</sub> ), 3.32 s (CH <sub>3</sub> )
carnitine	4.56 app q (7.0; <u>CHOH</u> ), 3.45-3.31 m (CH <sub>2</sub> N <sup>+</sup> ), 3.19 s ((CH <sub>3</sub> ) <sub>3</sub> N <sup>+</sup> ), 2.62-2.53 m (CH <sub>2</sub> COOH)
lipoic acid	3.62 m (H3), 3.20 m (H5α), 3.13 m (H5β), 2.46 sext (6.2; H4α), 2.30 t (7.4; H9), 1.91 sext (6.7; H4β), 1.72 m (H6α), 1.61 m (H6β, H8), 1.44 m (H7)
stearate	2.21 t (7.5; CH <sub>2</sub> CO), 1.53 quin (6.9; CH <sub>2</sub> CH <sub>2</sub> CO), 1.27 broad s (14 CH <sub>2</sub> ), 0.98 t (6.9; CH <sub>3</sub> )
linoleic acid	5.36 m (4 =CH), 2.78 t (6.6; =CH-CH <sub>2</sub> -CH=), 2.26 t (7.5; -CH <sub>2</sub> -COOH), 2.06 q (6.8; 2 -CH <sub>2</sub> -CH <sub>2</sub> -CH=), 1.55 quin (6.9; -CH <sub>2</sub> -CH <sub>2</sub> -COOH), 1.32 m (7 CH <sub>2</sub> ), 0.89 t (6.9; CH <sub>3</sub> )
sinapine <sup>d</sup>	7.65 d (15.9; Ar-CH=), 6.96 s (Ar), 6.48 d (15.9; =CH-COO-), 4.57 m (O-CH <sub>2</sub> ), 3.87 s (OCH <sub>3</sub> ), 3.68 m (-CH <sub>2</sub> -N(CH <sub>3</sub> ) <sub>3</sub> <sup>+</sup> ), 3.17 s (N(CH <sub>3</sub> ) <sub>3</sub> <sup>+</sup> )
citrate	2.75 AB system (17.3; CH <sub>2</sub> )
vitamin C	4.78 d (2.0; CH-O-), 3.89 td (X part of the ABX system) (2.0, 6.7; <u>CHOH</u> ), AB part of the ABX system: δ <sub>A</sub> = 3.62, δ <sub>B</sub> = 3.60 (J <sub>AB</sub> = 11.2, J <sub>AX</sub> = J <sub>BX</sub> = 6.7; <u>CH<sub>2</sub>OH</u> )
dehydroascorbic acid	4.58 s (CHO), 4.43 dd (5.6, 3.1; <u>CHOH</u> ), 4.21 dd (5.6, 10.2; 1H of CH <sub>2</sub> OH), 4.05 dd (3.1, 10.2; 1H of CH <sub>2</sub> OH)
vitamin B1 (thiamine)	9.56 s (H thiazole ring), 7.97 s (H pyrimidine ring), 5.47 s (CH <sub>2</sub> N <sup>+</sup> ), 3.82 t (5.6; <u>CH<sub>2</sub>OH</u> ), 3.13 t (5.6; CH <sub>2</sub> CH <sub>2</sub> OH), 2.61 s (CH <sub>3</sub> ), 2.50 s (CH <sub>3</sub> )
vitamin B3 (niacin)	8.98 d (2.1; H2), 8.70 dd (4.9, 1.7; H6), 8.23 td (8.0, 1.7; H4), 7.52 dd (4.9, 8.0; H5)
vitamin B5 (pantothenic acid)	3.90 s ( <u>CHOH</u> ), 3.46-3.34 m (CH <sub>2</sub> OH and CH <sub>2</sub> NHCO), 2.33 t (6.7; CH <sub>2</sub> COOH), 0.89 s (CH <sub>3</sub> ), 0.88 s (CH <sub>3</sub> )
vitamin B6 (pyridoxine)	8.08 s (H), 5.04 s (CH <sub>2</sub> OH), 4.67 s (CH <sub>2</sub> OH), 2.59 s (CH <sub>3</sub> )
vitamin E (tocopherol acetate)	2.62 t (6.8; =C-CH <sub>2</sub> -), 2.31 s (CH <sub>3</sub> -COO <sup>-</sup> ), 2.08 s (CH <sub>3</sub> -Ar), 1.99 s (CH <sub>3</sub> -Ar), 1.96 s (CH <sub>3</sub> -Ar), 1.82 m (O-C-CH <sub>2</sub> ), 1.53 m (HC-CH <sub>3</sub> ), 1.41 m (HC-CH <sub>3</sub> ), 1.27 m (CH <sub>2</sub> ), 1.25 s (CH <sub>3</sub> -C-O), 1.16 m (CH <sub>2</sub> ), 1.09 m (CH <sub>2</sub> ), 0.88 d (6.6; HC-(CH <sub>3</sub> )), 0.86 d (6.6; HC-CH <sub>3</sub> )

$\alpha/\beta$ -glucose	5.14 d (3.5; H1 $\alpha$ ), 4.53 d (8.0; H1 $\beta$ ), 3.84-3.71 m, 3.71-3.60 m, 3.45-3.28 m, 3.15 t (8.0; H2 $\beta$ )
fructose	4.06-3.99 m, 3.99-3.85 m, 3.85-3.68 m, 3.67-3.42 m
sucrose <sup>e</sup>	5.36 d (3.5; g1), 4.10 d (8.5; f3), 4.00 t (8.5; f4), 3.83-3.67 m (f5, g5, f6, g6, g3), 3.61 AB system (12.5; f1), 3.48 dd (3.5, 10.0; g2), 3.38 t (9.5; g4)

<sup>a</sup> Spectral data were measured from authentic standards in CD<sub>3</sub>CN:D<sub>2</sub>O (80:20).

<sup>b</sup> s: singlet; d: doublet; dd: doublet of doublet; t: triplet; td: doublet of triplet; q: quadruplet; quin: quintuplet; sext: sextuplet; sept: septuplet; m: multiplet; app: apparent.

<sup>c</sup> The chemical shifts were measured in the spectrum of formulation **19**.

<sup>d</sup> The structure of sinapine (choline ester of sinapic acid) was determined with 1D and 2D <sup>1</sup>H and <sup>13</sup>C NMR experiments on formulation **14** and confirmed by MS ([M<sup>+</sup>] 310).

<sup>e</sup> g: glucose; f: fructose.



Table 5. Amounts of adulterant sibutramine and phenolphthalein found in claimed herbal medicines analyzed in this study.

Formulation number	Sibutramine mg/capsule or tablet mean±SD (n = 3)	Phenolphthalein mg/capsule or tablet mean±SD (n = 3)
<b>1</b>	13.1 ± 0.3	47.0 ± 3.0
<b>2</b>	4.4 ± 0.3	-
<b>3</b>	23.3 ± 0.1	-
<b>4</b>	15.6 ± 0.5	-
<b>5</b>	19.6 ± 0.5	66.1 ± 1.4
<b>7</b>	5.0 ± 0.1	36.2 ± 0.5
<b>8</b>	12.7 ± 0.2	-
<b>9</b>	12.0 ± 0.2	4.4 ± 0.2
<b>10</b>	10.6 ± 0.3	-
<b>11</b>	30.5 ± 0.1	-
<b>13</b>	15.1 ± 0.3	-
<b>14</b>	14.0 ± 0.7	-
<b>15</b>	13.7 ± 0.6	40.8 ± 1.3

Table 6. Adulterants found in herbal products or dietary supplements marketed as slimming products reported in literature

References	Analytical methods for detection	Number of formulations analysed	Active Pharmaceutical Ingredients detected	Other compounds detected
Tseng et al. 2000	GC-MS	4	Mazindol Clobenzorex Diazepam Phentermine Caffeine	
Jung et al. 2006	GC-MS HPLC-DAD	1	Sibutramine	
Blachut et al. 2007	GC-MS	1 (Lida)	N-desmethyilsibutramine	
Sein Anand and Chodorowski 2007	GC-MS	1 (Meizitanc)	Sibutramine	
Bogusz et al. 2006	LC-ESI-MS/MS	1	Fenfluramine Phentermine Caffeine	
Zou et al. 2007	LC-MS/MS LC-TOF-MS	1	Sibutramine N-desmethyilsibutramine N-didesmethyilsibutramine Homosibutramine	
Date et al. 2008	LC-ESI-MS/MS	10	Phenolphthalein Sibutramine Mazindol Sennosides A and B Bisacodyl Hydrochlorothiazide	
Huang et al. 2008	LC-ESI-MS	15	Sibutramine N-didesmethyilsibutramine	
Wang et al. 2008	LC-ESI-MS	6	Sibutramine Phenolphthalein N-desmethyilsibutramine	
Chen et al. 2009	QTRAP LC-MS/MS	18	Fenfluramine Sibutramine Phenolphthalein	
Cianchino et al. 2008	CE	4	Ephedrine Norephedrine Furosemide Caffeine	
This study	<sup>1</sup> H NMR and 2D DOSY <sup>1</sup> H NMR	20	Sibutramine Phenolphthalein Synephrine Methylsynephrine Vitaberin Caffeine	Carnitine Vitamin C Dehydroascorbic acid Vitamins B1, B3, B5, B6, E Glucose Fructose Sucrose Stearate Linoleate Citrate Hydroxycitrate Tartrate Sinapine